Remarks

In accordance with the present invention, there are provided methods for testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR-γ). Invention methods comprise assaying for changes in the level of reporter protein present as a result of contacting cells containing PPAR-γ and a reporter vector with the compound of interest. Compounds identified employing invention methods are useful in the treatment of pathological conditions such as diabetes.

Claims 16-20 and 22-28 were pending before this communication. Claims 22-26 have been withdrawn from consideration by the Examiner pursuant to Applicants' election (with traverse) of Group II (claims 16-20, 27 and 28). By this response, claims 16, 20, 27 and 28 have been amended to define Applicants' invention with greater particularity, and non-elected claims 22-26 have been cancelled without prejudice.

Also by this response, the specification has been amended to correct obvious typographical errors in the sequence identifiers (SEQ ID NOs) and conform the specification to the previously filed sequence listing. In addition, a replacement Abstract is also provided herein.

These amendments add no new matter and are fully supported by the specification and the original claims. Applicants respectfully submit that the amendments presented herein place the application in condition for allowance or, at a minimum, reduce the issues for appeal. Accordingly, entry of the amendments is respectfully requested. Attached hereto is a marked-up version of the changes made to the specification and the claims, labeled APPENDIX A.

Accordingly, claims 16-20, 27 and 28 are currently pending. For the Examiner's convenience, a clean copy of all claims is provided in APPENDIX B.

The objection to the specification on the grounds that the application allegedly does not contain an abstract is respectfully traversed. In Applicants' response mailed December 14, 2001, an Abstract of the Disclosure was submitted (see Paper No. 19 at page 10). Since this objection is repeated herein, Applicants' previous efforts to address this issue have apparently been unsuccessful. Therefore, in further efforts to expedite prosecution and to reduce the issues, a copy of the original Abstract of the Disclosure is provided herein (page 40 of the specification as filed) on a separate sheet of paper with no additional text. Accordingly, Applicants again request reconsideration and withdrawal of this objection to the specification.

The rejection of claims 16-20, 27 and 28 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains to make and/or use the invention, is respectfully traversed. The specification clearly enables one of skill in the art to make the PPAR-γ receptor and the reporter vector and cells containing these components, and to use the resulting cells to test compounds for their ability to regulate PPAR-γ effects.

(1) Enablement with respect to Claims 16-19

Invention methods, as defined by claim 16 (and claims dependent thereon), require contacting cells, which contain both a PPAR-γ receptor and a reporter vector, with a test compound; and assaying for changes in the level of reporter protein thereby determining the ability of the test compound to regulate transcription-activation effects of the PPAR-γ receptor. The reporter vector comprises three elements: (a) a promoter that is operable in the cell; (b) a hormone response element; and (c) a DNA segment encoding the reporter protein to be assayed. Each element contemplated by claims 16-19 is clearly enabled by the specification as detailed below. Indeed, the Examiner has acknowledged that "Examples 2-3 describe the preparation of various reporter constructs and screening assays and one skilled in the art would be familiar with these preparation and screening methods" (emphasis added, see Office Action, Paper No. 20, at page 5, lines 6-7).

Thus, the Examiner's assertion that "the skilled artisan would not know the elements encompassed by the pTK-MH100x4-LUC [reporter] construct utilized in Examples 2-3" (see Office Action, Paper No. 20, at page 5, lines 8-9), is respectfully submitted to be without merit. Contrary to the Examiner's assertion, each of the three elements of the reporter vector as outlined above are clearly presented in the specification.

For example, the promoter element of the reporter vector, (a), is clearly described in the specification. "Exemplary promoters include the simian virus (SV) promoter or modified form thereof (e.g., ΔSV), the thymidine kinase (TK) promoter, the mammary tumor virus (MTV) promoter or modified form thereof (e.g., ΔMTV), and the like" (see specification at page 15, line 32, through page 16, line 4). In the working examples pointed to by the Examiner, the reporter vector pTK-MH100x4-LUC would clearly be understood by one of skill in the art to comprise the TK promoter, designated "pTK" in the vector name.

Similarly, the hormone response element of the reporter vector, (b), is clearly described in the specification.

> Hormone response elements contemplated for use in the practice of the present invention are composed of at least one direct repeat of two or more half sited separated by a spacer of one nucleotide. The spacer nucleotide can be selected from any one of A, C, G or T. Each half site of response elements contemplated for use in the practice of the invention comprises the sequence -RGBNNM-, wherein R is selected from A or G; B is selected from G, C, or T; each N is independently selected from A, T, C, or G; and M is selected from A or C; with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence -AGGTCA-.

(See specification at page 13, lines 13-29).

Additional characteristics of response elements and citations to exemplary response elements are also provided in the specification.

> Exemplary PPREs have been described in detail hereinabove. Exemplary GAL4 response elements are those containing the palindromic 17-mer: 5' - CGGAGGACTGTCCTCCG - 3' (SEQ ID NO:7) . . . as well as derivatives

thereof. Additional examples of suitable response elements include those described by Hollenberg and Evans in Cell 55:899-906 (1988); or Webster et al. in Cell 54:199-207 (1988).

(See specification at page 15, lines 20-29).

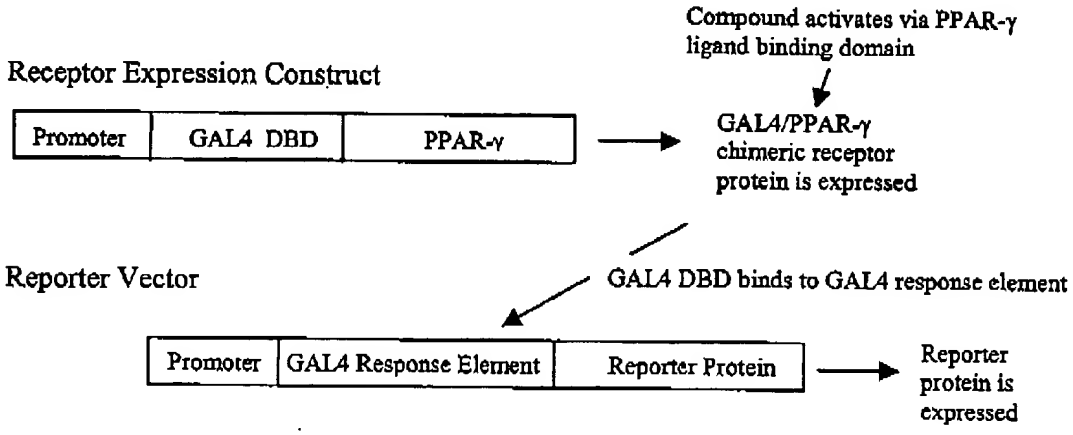
Likewise, the reporter protein-encoding segment of the reporter vector, (c), is clearly described in the specification. "Exemplary reporter genes include chloramphenicol transferase (CAT), luciferase (LUC), beta-galactosidase (β-gal), and the like." (see specification at page 15, lines 30-32). The specification further teaches that the reporter vector, in Examples 2 and 3 the pTK-MH100x4-LUC, "can be any plasmid which contains an operative PPRE or GAL4 response element, as appropriate, functionally linked to an operative reporter gene". In these examples, the reporter employed is luciferase (commonly referred to as LUC) (see specification at page 15, lines 17-19).

The Examiner's further assertions that "there is a lack of guidance in the specification regarding whether or not the pTK-MH100x4-LUC construct contains any hormone response elements" and that "undue experimentation would be required of the skilled artisan to determine the specific sequence and the number of repeats of the hormone response element" (see Office Action, Paper No. 20, at page 5, lines 9-12) indicate an incomplete understanding of Applicants' invention. In addition to the guidance in the specification explained above, the specification describes the response element component of the reporter construct as a segment of DNA that is positioned upstream of the promoter and the reporter gene and which provides the sequence to which the DNA binding domain of the receptor specifically binds. The specification also indicates that "the reporter gene will be expressed as the result of the fact that the 'PPRE' or 'GAL4 response element' was 'turned on' or otherwise activated (see specification, at page 16, lines 21-31). Thus, the response element in the reporter construct is clearly described as either the hormone response element, PPRE, or the GAL4 response element. One of skill in the art would know that these two elements provide analogous functions, and further, one of skill in the

art would be able to determine, depending on the receptor, e.g., PPAR-γ or chimeric GAL4/PPAR-γ, which element to utilize.

Moreover, Example 2 describes how to make various reporter constructs for use in methods of the present invention. For example, the construct pTK-PPRE3-LUC contains three copies of PPRE oligonucleotides upstream of the TK promoter driving expression of the luciferase reporter (see specification, at page 23, lines 14-17). Similarly, the description of the pTK-MH100x4-LUC construct in Example 2 explicitly states "four copies of double-stranded MH100 oligonucleotides, encoding a GAL4 binding site" were used in the preparation of the construct (emphasis added, see specification at page 23, lines 18-20). The abbreviation MH100x4 clearly refers to four copies of the MH100 oligonucleotide, encoding four copies of the GAL4 binding site. Again, the choice of the appropriate combination of response element is easily made by one skilled in the art to correspond to the receptor being used in the expression system. Thus, the identity of the response element of the pTK-MH100x4-LUC construct and the specific sequence and number of repeat elements are clearly described in the specification.

The specification also provides a complete exemplary screening assay for PPAR-γ-selective agonists (as contemplated by claim 16) in Example 3. Specifically, this exemplary assay utilizes cells comprising both a PPAR-γ receptor (introduced by way of a receptor expression construct) and a reporter vector (comprising (a) a promoter, (b) a GAL4 response element, and (c) a reporter protein), to test compounds for their ability to regulate PPAR-γ transcription-activation of the reporter vector, by monitoring the level of reporter protein. The GAL4 response element is used here because the screening assay is performed with a chimeric PPAR-γ receptor (CMX-GAL-PPARγ), which requires the GAL4 response element as its DNA binding site (see, for example, specification at page 14, lines 20-29), as illustrated below.



Furthermore, as acknowledged by the Examiner, "Example 3 teaches co-transfecting CV-1 cells with CMX-GAL-PPAR-γ [the receptor construct] and pTK-MH100x4-LUC [the reporter vector] and incubating the cells with various agonists [test compounds]" (see Office Action, Paper No. 20, at page 5, lines 13-15). This is all that is required in order to carry out the invention method of independent claim 16. One of skill in the art could clearly make and use a complementary pair of receptor and reporter constructs by following the teachings of the specification. Therefore, invention methods testing a single compound, as contemplated in claims 16-19, are clearly enabled by the specification as filed. The specification provides more than a reasonable amount of guidance, specifically describing all components of the constructs contemplated, and their use in invention assays. Accordingly, one of skill in the art could readily make and use the invention as described in claims 16-19.

(2) Enablement with Respect to Claim 20

Invention methods, as defined by claim 20, further require that the test compound (a putative antagonist) is assayed at increasing concentrations in the presence of a fixed concentration of at least one PPAR-γ agonist. By definition, an antagonist attenuates the effect of an agonist. Therefore, in order to determine whether a test compound is a putative antagonist, the presence of an agonist is required to provide a base-line level of effect for the agonist, thereby permitting one to determine the effect of the antagonist. Assays employing the

combination of an agonist with a test compound are typically performed using concentration gradients to determine whether the test compound is an antagonist. These methods are standard in the field of pharmacology when discerning the effects of test compounds on any target receptor (see, for example, Kenakin and Kenakin, *Molecular Pharmacology: A Short Course*, Blackwell Science, Inc., 1997). Applicants need not describe in detail what is already well known in the art.

The Examiner's assertions that "the specification does not teach contacting the cells with at least one antagonist" or "with a fixed concentration of agonist concomitantly with increasing concentrations of any compound" (see Office Action, Paper No. 20, at page 5, lines 15-18) are clearly in error. Contrary to the Examiner's assertions, the specification specifically describes contacting cells with both an agonist and a test compound at increasing concentrations to determine the ability of the test compound to inhibit activation of transcription by the PPAR-γ agonist (i.e., to function as a PPAR-γ antagonist). See, for example, specification at page 18, line 11, through page 19, line 6:

> In accordance with another embodiment of the present invention, there is provided a method of screening for antagonists of PPARγ receptor proteins, said method comprising culturing test cells containing (i) increasing concentrations of at least one compound whose ability to inhibit the transcription activation activity of PPARγ agonists is sought to be determined, and (ii) optionally, at least one PPARγ agonist, wherein said test cells contain [PPARγ and a reporter vector]; and thereafter assaying for evidence of transcription of said reporter gene in said cells as a function of the concentration of said compound in said culture medium, thereby indicating the ability of said compound to inhibit activation of transcription by PPARγ agonists.

This is all that is required in order to carry out the invention method of claim 20. Therefore, invention methods testing two compounds, wherein one is a known agonist and the other is a putative antagonist, as contemplated by claim 20, are clearly enabled by the specification as filed. The specification provides more than a reasonable amount of guidance, specifically describing all components of the constructs contemplated, and their use in invention assays. Accordingly, one of skill in the art could readily make and use the invention as described in claim 20.

(3) Enablement with Respect to Claims 27 and 28

Invention methods, as defined by claims 27 and 28, further require that the test compound is assayed in the presence of at least one additional compound, a PPAR-γ agonist (claim 27) or a PPAR-γ antagonist (claim 28). As mentioned above, methods of combining potential receptor agonists and/or antagonists are standard in the field of pharmacology when discerning the effects of test compounds on any target receptor.

For example, in determining the efficacy and mode of function (i.e., receptor agonist or antagonist) of a test compound, a variety of concentration curves are typically generated. Exemplary assays might include determining receptor response in the presence of a test compound and various concentrations of a second compound, to be compared with receptor response in the presence of one compound alone. An increased response in the presence of an agonist in addition to the test compound, as compared to the agonist alone, would indicate that the test compound is an additive or synergistic agonist. A decreased response in the presence of an agonist in addition to the test compound, as compared to the agonist alone, would indicate that the test compound is an antagonist. A decreased response in the presence of an antagonist in addition to the test compound, as compared to the test compound alone, would indicate that the test compound is an agonist. Other potential combinations of compounds for pharmacological screening of the PPAR-γ receptor are known to one of skill in the art, and would clearly be recognized as effective methods of testing a test compound for its ability to regulate PPAR-γ. As quoted above, the specification clearly contemplates combining compounds to evaluate the ability of a test compound to regulate PPAR-γ transcription-activating effects. Accordingly, one of skill in the art could readily make and use the invention as described in claims 27 and 28.

(4) Enablement with Respect to Test compounds

Applicants respectfully disagree with the Examiner's assertion that "the specification provides little guidance regarding what sort of compounds should be screened" (see Office Action, Paper No. 20, at page 5, lines 19-20). Contrary to the Examiner's assertion, a multitude

of exemplary compounds, including PPAR-γ selective prostaglandin or prostaglandin-like compounds, are described in the specification.

> PPAR-γ-selective prostaglandins or prostaglandin-like compounds contemplated for use in the practice of the present invention include members of the prostaglandin-J2 family of compounds . . .members of the prostaglandin-D2 family of compounds . . . or precursors thereof, as well as compounds having the structure I.

(See specification at page 4, lines 19-27). In addition to exemplary compounds, working compounds are also presented in Example 1, and Figures 1 and 2. The specification further provides that test "compounds can be readily prepared using a variety of synthetic methods, as are well known by those of skill in the art. For example . . . chemically or enzymatically, from the naturally occurring precursor, arachidonic acid" (see specification at page 11, lines 9-14). Accordingly, one of skill in the art could readily identify appropriate classes of test compounds to use in the methods of the present invention.

For all of the reasons cited above, it is respectfully submitted that Applicants have clearly set forth how to make and use the present invention as required by 35 U.S.C. § 112, first paragraph. In summary, minimal routine experimentation is necessary to determine the sequence of an appropriate response element in light of the extensive direction presented in the specification regarding the response elements, as well as regarding the entire receptor and reporter constructs. The specification provides working examples of invention methods comprising one test compound, and clearly contemplates practicing such methods in the presence of additional compounds according to standard pharmacological techniques. The breadth of the claims is commensurate with the disclosure provided by the specification. Thus, it is clear that those skilled in the art would not require undue experimentation to practice the claimed invention. Accordingly, reconsideration and withdrawal of this rejection under 35 U.S.C. § 112, first paragraph, are respectfully requested.

The rejection of claims 16-20, 27 and 28 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite is respectfully traversed. Applicants respectfully submit that the

claims as written particularly point out and distinctly claim the subject matter which Applicants regard as their invention.

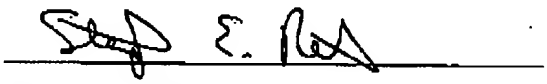
However, in order to expedite prosecution and to reduce the issues, claims 16, 20, 27 and 28 have been amended to further clarify the relationship of the steps to the recited preamble as suggested by the Examiner (see Office Action, Paper No. 20, at page 6, lines 18-21). Accordingly, reconsideration and withdrawal of this rejection under 35 U.S.C. § 112, second paragraph, are respectfully requested.

Conclusion

In view of the above amendments and remarks, reconsideration and favorable action on all claims are respectfully requested. In the event any matters remain to be resolved in view of this communication, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

Date: July 26, 2002



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Enclosures: **Appendices A and B**
Abstract of the Disclosure (page 40 of the original specification as filed)

APPENDIX A – ALTERED SPECIFICATION AND CLAIMS

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

The paragraph beginning at line 33 of page 13 has been amended as follows:

--Presently preferred response elements contain at least one copy (with one, two or three copies most common) of the minimal sequence:

AGGACA A AGGTCA (SEQ ID NO:5[4]).

As noted above, the minimal sequence can optionally be flanked by additional residues, for example, as in the sequence:

GGACC AGGACA A AGGTCA CGTTC (SEQ ID NO:6[5]).--

The paragraph beginning at line 20 of page 15 has been amended as follows:

-- Exemplary PPREs have been described in detail hereinabove. Exemplary GAL4 response elements are those containing the palindromic 17-mer:

5' - CGGAGGACTGTCCTCCG - 3' (SEQ ID NO:7[6]),

such as, for example, 17MX, as described by Webster et al., in Cell 52;169-178 (1988), as well as derivatives thereof. Additional examples of suitable response elements include those described by Hollenberg and Evans in Cell 55;899-906 (1988); or Webster et al. in Cell 54:199-207 (1988). --

The paragraph beginning at line 30 of page 21 has been amended as follows:

-- A basic vector useful for the generation of GAL4-receptor fusion proteins is called pCMX-GAL4 (see SEQ ID NO:3[2]). This vector encodes GAL4 DNA binding domain, followed by a polylinker sequence useful in the cloning. The parental expression vector pCMX has been described by Umesono et al., in Cell 65:1255-1266 (1991), and the GAL4 portion of pCMX-GAL4 is derived from plasmid pSG424, described by Sadowski and Ptashne, in Nucleic Acids Res. 17:7539 (1989). --

The paragraph beginning at line 14 of page 23 has been amended as follows:

-- pTK-PPRE3-LUC: Three copies of double-stranded peroxisome proliferator response element (PPRE) oligonucleotides (see SEQ ID NO:5[3]) were cloned upstream of the TK promoter of TK-LUC at the *SalI* site. --

In the claims:

Claims 16, 20, 27 and 28 have been amended as follows:

16. (Amended) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR-γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing said receptor and reporter vector with said compound;

wherein said reporter vector comprises:

(a) a promoter that is operable in said cell,

(b) a hormone response element, and

(c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof,

wherein an increase or decrease in the level of the reporter protein detected when said cells are contacted with said compound, relative to the level of the reporter protein detected when said cells are not contacted with said compound, is indicative of a compound that regulates the transcription-activating effects of said receptor.

20. (Amended) A method according to claim 16 wherein said compound is a putative antagonist for said peroxisome proliferator activated receptor-gamma, and wherein said contacting is carried out in the presence of

increasing concentrations of said compound, and

a fixed concentration of at least one agonist for said peroxisome proliferator activated receptor-gamma,

wherein a decrease in the level of the reporter protein detected when said cells are contacted with said compound and said agonist, relative to the level of the reporter protein detected when said cells are contacted with said agonist alone, is indicative of a compound that is an antagonist of said receptor.

27. (Amended) A method according to Claim 16 wherein said contacting is carried out in the further presence of at least one PPAR-γ agonist,

wherein an increase or decrease in the level of the reporter protein detected when cells are contacted with said compound and said agonist, relative to the level of the reporter protein detected when cells are contacted with said agonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

28. (Amended) A method according to Claim 16 wherein said contacting is carried out in the further presence of at least one PPAR-γ antagonist,

wherein an increase or decrease in the level of the reporter protein detected when cells are contacted with said compound and said antagonist, relative to the level of the reporter protein detected when cells are contacted with said antagonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

Claims 22-26 have been cancelled without prejudice.

APPENDIX B – CURRENTLY PENDING CLAIMS

16. (Amended) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR-γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing said receptor and reporter vector with said compound;

wherein said reporter vector comprises:

(a) a promoter that is operable in said cell,

(b) a hormone response element, and

(c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof,

wherein an increase or decrease in the level of the reporter protein detected when said cells are contacted with said compound, relative to the level of the reporter protein detected when said cells are not contacted with said compound, is indicative of a compound that regulates the transcription-activating effects of said receptor.

17. A method according to Claim 16 wherein said hormone response element is a direct repeat of two or more half sites separated by a spacer of one nucleotide, wherein said spacer can be A, C, G or T, wherein each half site comprises the sequence

-RGBNNM-,

wherein

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence -AGGTCA-; and

wherein said response element is optionally preceded by N_x, wherein x falls in the range of 0 up to 5.

18. (Amended) A method according to claim 17 wherein said response element has at least one copy of the minimal sequence:

AGGACA A AGGTCA (SEQ. ID NO. 5),

wherein said minimal sequence is optionally flanked by additional residues.

19. (Amended) A method according to claim 17 wherein said response element has at least one copy of the sequence:

GGACC AGGACA A AGGTCA CGTTC (SEQ. ID NO. 6).

20. (Amended) A method according to claim 16 wherein said compound is a putative antagonist for said peroxisome proliferator activated receptor-gamma, and wherein said contacting is carried out in the presence of

increasing concentrations of said compound, and

a fixed concentration of at least one agonist for said peroxisome proliferator activated receptor-gamma,

wherein a decrease in the level of the reporter protein detected when said cells are contacted with said compound and said agonist, relative to the level of the reporter protein detected when said cells are contacted with said agonist alone, is indicative of a compound that is an antagonist of said receptor.

27. (Amended) A method according to Claim 16 wherein said contacting is carried out in the further presence of at least one PPAR-γ agonist,

wherein an increase or decrease in the level of the reporter protein detected when cells are contacted with said compound and said agonist, relative to the level of the reporter protein detected when cells are contacted with said agonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

28. (Amended) A method according to Claim 16 wherein said contacting is carried out in the further presence of at least one PPAR-γ antagonist,

wherein an increase or decrease in the level of the reporter protein detected when cells are contacted with said compound and said antagonist, relative to the level of the reporter protein detected when cells are contacted with said antagonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.